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# DESIGN AND OPERATION OF A SMALL TANK SYSTEM FOR OVARIAN MATURATION AND SPAWNING OF *PENAEUS VANNAMEI*

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*ABSTRACT* Ovarian maturation and spawning of *Penaeus vannamei* was accomplished in a 120 l (30 gal) tank as well as in a 800 l recirculating system consisting of six aquaria plumbed into a common biofilter. Pond reared animals stocked one per tank were fed a diet consisting of commercial pellets, squid, and bloodworms. Temperature was maintained at 28°C and salinity between 28 and 32 ppt. Presence of black shields between aquaria, males, eggs in the water, and other females in the aquaria were not required for ovarian maturation. Thirty-six of 78 females held in the small tank system spawned. The smallest female which spawned was 25.9 g. After unilateral eyestalk ablation by enucleation, female *P. vannamei* molted in 3-16 days and spawned in 7-20 days. Up to four spawns occurred before the next molt which occurred 15-35 days after ablation.

## Introduction

The white legged shrimp of the Central American Pacific Coast, *Penaeus vannamei* or "vanna whites," have become the species of choice for culture in the Americas. One recurring problem in the aquaculture of this species has been the variable seed supply. Postlarval abundance off Ecuador has determined the economics for both wild and hatchery produced seedstock. The yearly abundance of postlarvae off Ecuador is determined by ocean temperatures linked to the "El Niño" for which no predictive models are available. Maturation of *P. vannamei* in captivity is difficult but obtainable (Boeing 1988). Production of this shrimp species is low due to low mating success, low egg fertilization and a low hatching rate (Boeing 1988). Finally, there is a perception among commercial farmers that the quality of hatchery produced postlarvae is not as good as wild larvae and consequently, they sell for less (Montealegre 1989). Commercially, the majority of maturation tanks are over 3.8 m (15 ft) in diameter (Ogle 1991) and due to a requirement for feeding fresh feeds, operation of a maturation facility can be expensive. Research on ovarian maturation of marine penaeid shrimp has been limited due to the time, cost and difficulty of replication associated with large tank systems. Few research institutions have the facilities to accomplish shrimp maturation and few commercial facilities are willing to conduct replicated experiments in production tanks. For these reasons, a closed recirculating system was developed using small tanks to experimentally determine factors influencing ovarian maturation of *P. vannamei*. The objective was to conduct replicable experiments with minimal space and expense for a large number of factors that might influence egg production and egg quality. It is recognized that studies on mating will still require large tanks (Ogle 1991), although *in vitro* fertilization might be accomplished with the small tank systems. Finally, the small tank system allows close control of individual animals, controlled breeding and pre-screening of animals for reproductive performance.

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## Materials and Methods

The system (Fig. 1) consisted of six 120 l (30 gal) aquaria plumbed in common to an external trickling biofilter. A 2.54 cm (1 in) hole was drilled in the upper left corner of one end of each aquaria to provide an overflow. A thruhull fitting was fabricated from a polyvinyl chloride (PVC) male adaptor and a PVC (slip threaded slip) threaded through the hole in the aquarium. A 15 cm (6 in) length of 2.54 cm (1 in) PVC pipe having a number of 1.27 cm (1/2 in) holes was wrapped with fiberglass window screen secured with silicone sealant. This pipe, capped on one end and inserted into the male adaptor, constituted an internal screened overflow. A length of PVC pipe directed the water from the overflow down through an egg collector into a common collection trough. The egg collectors were fabricated from plastic 473 ml (16 oz) beverage bottles. The bottles had 5.08 cm (2 in) square holes cut in the side and covered with 180 micron nitex secured with silicone sealant. Waste water from the trough cascades into a downflow submerged trickling filter. The filter consisted of a 83 l (22 gal) white, round plastic trash container filled with a filter

media of clam shells (*Rangia* sp.). A 20 cm (8 in) PVC pipe extended from the bottom of the container to just above the top of the container. This pipe housed a submersible pump (Little Giant, Oklahoma City, OK, Model NK-1) which provided filtered water through a 1.27 cm (1/2 in) vinyl hose to an overhead manifold. The 213 cm (7 ft) manifold was constructed of 10 cm (4 in) PVC pipe capped at both ends. A thruhull fitting of 3.8 cm (1 1/2 in) PVC was constructed from a male adaptor and a tee inserted into one end cap to provide for overflow of the manifold. Excess water was directed back into the top of the trickling filter by a length of PVC pipe. Two 2.54 cm x 91 cm (1 in x 3 ft) slots were cut into the top of the manifold pipe. The slots allowed positioning of air line tubing which was used as siphons to supply water to each aquaria. A piece of 1.9 cm (3/4 in) PVC pipe filled with sand and capped at both ends to which the air line tubing was attached with plastic wire-ties was used as a weight to secure the siphons in the manifold. Each aquarium received water having a flow rate of 690 ml/min. Each aquarium was aerated with a single airstone and covered with plexiglass. A plastic clothespin was glued to the top of the covers to secure cards used to record data for each tank.

Settled estuarine water of at least 18 ppt was evaporated to 32 ppt by heating to 80°C and provided the natural seawater for the system. Total volume of the entire system was approximately 1100 l (300 gal). Groups of six aquaria were contained in a common water table 214 cm x 106 cm x 15 cm (84 in x 42 in x 6 in) deep filled with 287 l (75 gal) of freshwater. Water temperature was generally maintained by regulating room air temperature. Additionally, each water table was equipped with a thermostatically controlled 100-watt titanium heater (Glow-Quartz, Mentor, Ohio). A submersible pump (Little Giant Model p-AAA) positioned at the opposite end of the water table from the heater provided water circulation to ensure equal temperature distribution. Six complete systems of six tanks each were located in a 3 m x 9.4 m room. Lighting was provided by eight 40-watt cool white fluorescent bulbs located overhead in the room. A mechanical timer controlled the lights and was

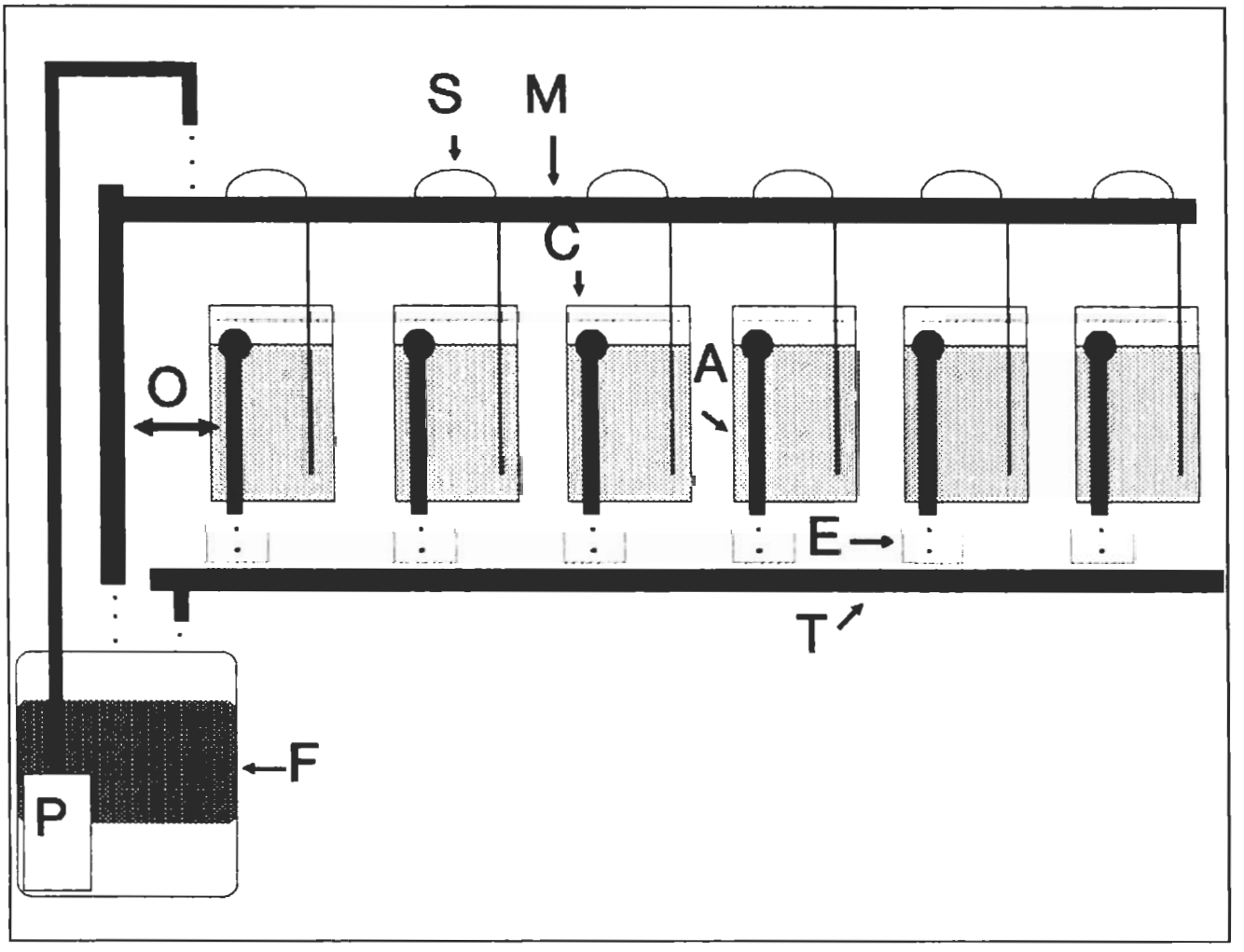


**Figure 1. A cross-section diagram of the small tank system used to achieve ovarian maturation and spawning of *Penaeus vannamei:* S siphon, M manifold, C cover, A aquaria, E egg collector, F filter, P pump, T trough, and O overflow.**

used to provide a photoperiod of 14L:10D hours.

Initial efforts to achieve ovarian maturation of *P. vannamei* involved the use of black shields between aquaria, placing males in the system, placing two females in each aquaria and adding shrimp eggs from another tank to each system. An additional study investigated the effect of eyestalk ablation 5 and 10 days after molting. The left eyestalk was ablated by enucleation. In a final experiment of five separate trials, an artificial seawater mix (Marine Environment, San Francisco, CA) was compared to natural sea water. A reverse osmosis filter (Nimbus, San Diego, CA) was used to purify the well water for mixing with artificial sea salt.

Water samples were analyzed weekly for pH, total ammonia, nitrite, nitrate, temperature and salinity. Ammonia and pH were determined by using an Orion pH/ion analyzer with appropriate electrode. Nitrite and nitrate levels were determined by titration (EPA 1983). Salinity and temperature were determined with a refractometer and mercury thermometer, respectively.

A standard management procedure involved the replacement of filter media for each study, cleaning the system and refilling with freshly filtered well water. Artificial salt was mixed and allowed to circulate at least three days before animals were stocked. Female *P. vannamei* of at least 30 g body weight that have never undergone ovarian maturation were used. A commercial shrimp grower pellet was fed until the animals molted, at which time the shrimp were started on a maturation diet consisting of squid in the morning, bloodworms at noon and maturation pellets in the evening. The left eyestalk was ablated five days after their first molt. Tanks were cleaned daily by siphoning and replacing part of the water. Egg collectors were added in the evenings commencing with the ablation of the animal and checked for eggs the following morning. If spawning did not occur, animals were discarded after their fourth molt.

*(Use of trade names does not imply endorsement.)*

## RESULTS

Water temperature during the initial studies was maintained at 28°C and salinity between 28-32 ppt. Total ammonia ranged from 0.01 ppm to 0.22 ppm, pH ranged from 8.16 to 7.41, nitrite peaked at 1.12 ppm and nitrate increased to a high of 118.4 ppm.

Presence of black shields between aquaria, males, eggs in the water and other females in the aquaria were not required for maturation. Of the 27 animals held in the preliminary study, 13 spawned while not subjected to the above conditions. Twelve animals either died or were lost from jumping out of the tanks; the remaining animals never spawned. The average size of the females that did not spawn was 35.7 g, the average size of the females that did spawn was 38.2 g and the smallest female that spawned was 25.9 g. Five of the 10 animals that were ablated five days after molting spawned. Eight of the 15 animals that were ablated ten days after molting spawned. After unilateral eyestalk ablation, female *P. vannamei* molted in 3-16 days and spawned in 7-20 days. Up to four spawns occurred before the next molt which occured 15-35 days after eyestalk ablation. In five subsequent studies (Table 1), 19 of 29 females spawned in artificial seawater and 14 of 22 spawned in natural estuarine water. Spawning occurred an average of 12.4 days past eyestalk ablation for shrimp maintained in artificial seawater and 9.3 days past ablation for shrimp maintained in natural estuarine water. In the artificial seawater, 31.6% of the females spawned within the same molt cycle that ablation occurred, whereas 64.3% of the animals held in natural water spawned in the same cycle. The second molt occurred in 13.4 days for both groups.

## DISCUSSION

This is the first account of which the author is aware for the maturation and spawning of *P. vannamei* in a small tank system utilizing artificial seawater and recirculation. Maturation of the penaeid shrimp, *P. monodon*, has been recorded (Beard and Wickins 1980) for a small tank and spawning of *P. merguiensis* was accomplished in 32 l of water (Beard et al. 1977). Caillouet (1973) achieved ovarian development of *P. duorarum* in a 300 l system. Maturation and spawning of a single female, *P. vannamei*, was accomplished at a commercial venture (King James Shrimp) in large tanks utilizing an artificial seawater (Bob Brick, personal communication) in 1977. A majority of commercial maturation facilities utilize some recirculation of water in large tanks averaging over 3.6 m (15 ft) in diameter (Ogle 1991). Tanks used for penaeid shrimp maturation can range from 500 l to 50 $m^3$ (Primavera 1984; Muthu and Laxminarayana 1982). The system described here, patterned after a system used for production of mysid shrimp for bioassay, (Burke and Walker 1988) enables relatively inexpensive replication of maturation experiments under controlled conditions.

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## TABLE 1

**Comparison of reproductive performance of female *P. vannamei* maintained in artificial seawater and natural seawater.**

| | Marine Environment | | | | Pure Bay Water | | |
|---|---|---|---|---|---|---|---|
| System Number | 1 | 2 | 3 | Avg. | 1 | 2 | Avg. |
| Females/System | 9 | 10 | 10 | 29 | 13 | 9 | 22 |
| % Spawn | 88.9 | 60 | 50 | 65.6 | 75 | 50 | 63.7 |
| % No Spawn | 0 | 0 | 30 | 10.3 | 8.3 | 0 | 4.5 |
| % Dead | 11.1 | 40 | 20 | 24.1 | 16.7 | 50 | 31.8 |
| Spawn DPA* | 11.4 | 14.8 | 11 | 12.4 | 8.2 | 10.4 | 9.3 |
| **PER CENT SPAWN DURING MOLT CYCLE** | | | | | | | |
| Cycle 1 | 75 | 16.7 | 60 | 31.6 | 66.7 | 60 | 64.3 |
| Cycle 2 | 25 | 66.7 | 40 | 63.1 | 33.3 | 40 | 35.7 |
| Cycle 3 | --- | 16.6 | --- | 5.3 | 0 | 0 | 0 |
| **INTERMOLT DURATION IN DAYS** | | | | | | | |
| Cycle 1 | 12.5 | 13.2 | 14.6 | 13.4 | 13.8 | 10.4 | 13.4 |
| Cycle 2 | --- | 13.0 | 16.3 | 15.5 | 33 | --- | --- |
| Cycle 3 | --- | --- | 20.3 | 20.3 | 15 | --- | --- |
| **WATER QUALITY** | | | | | | | |
| **pH** | | | | | | | |
| Minimum | 7.73 | 7.78 | 7.93 | | 7.61 | 7.69 | |
| Maximum | 7.94 | 8.05 | 8.02 | | 8.07 | 7.95 | |
| **TOTAL AMMONIA (ppm)** | | | | | | | |
| Mimimum | 0.0205 | 0.0092 | 0.0075 | | 0.002 | 0.0182 | |
| Maximum | 0.0483 | 0.0724 | 0.108 | | 0.183 | 0.347 | |
| **NITRITE (ppm)** | | | | | | | |
| Minimum | 0.0183 | 0.0138 | 0.0382 | | 0.00363 | 0.0286 | |
| Maximum | 0.0749 | 0.0957 | 1.67 | | 0.188 | 0.493 | |
| **NITRATE (ppm)** | | | | | | | |
| Minimum | 5.74 | 1.71 | 0.62 | | 1.15 | 0.10 | |
| Maximum | 19.9 | 21.7 | 22.5 | | 39.9 | 16.2 | |

*days past ablation

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